LOW TEMPERATURE ACCLIMATION IN THE DESERT SPIDER, *AGELENOPSIS APERTA* (ARANEAE, AGELENIDAE)

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ABSTRACT

Agelenopsis aperta (Gertsch) inhabits desert grasslands and lava beds in the southwestern U.S.A. The capacity of this species to cold-harden was assessed by exposing second generation laboratory-reared specimens to an artificial low temperature cycle simulating the "summer-autumn-winter" transition. Low temperature acclimation had no effect on whole body supercooling points, freeze tolerance or rates of oxygen consumption. Elevated levels of cryoprotectants were not detected using high performance liquid chromatographic techniques. Cold tolerance was similar between males, females and immatures. Exposure to temperatures immediately above the whole body supercooling point caused no apparent injury. It is hypothesized that movement into protected overwintering microhabitats may obviate the necessity for the evolution of seasonal mechanisms of cold-hardening in *A. aperta*.

INTRODUCTION

Cold-hardening refers to physiological mechanisms by which organisms acquire enhanced tolerance to low temperature. Few workers have examined the overwintering biology of arachnids, despite the fact that for temperate species winter represents a significant portion of the life span.

Comparable studies with terrestrial insects have revealed two basic patterns of cold-hardening (Salt 1961). One group tolerates the formation of extracellular ice within the body, whereas, other species are freeze susceptible or freezing intolerant. The latter avoid the lethal effects of freezing by lowering the temperature at which the spontaneous freezing (nucleation) of body water occurs. This value is termed the supercooling point and is experimentally determined by detecting the released latent heat of fusion as body water freezes. For intolerant species, the supercooling point represents the temperature at which body tissues freeze and the lower lethal limit.

Exposure to low environmental temperatures often serves as a proximal cue triggering the seasonal acquisition of increased cold tolerance (Baust 1981, Baust and Lee 1982). For both freeze susceptible and freeze tolerant arthropods cold-hardening is often associated with the accumulation of low molecular weight sugars (glucose and trehalose) and polyhydric alcohols (glycerol, sorbitol and mannitol). These cryoprotectants are believed

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to afford protection in a number of ways including the colligative depression of super-cooling and melting points, a reduction in the rate and amount of tissue ice formation and by the prevention of injurious levels of cellular dehydration (Meryman 1974).

The purpose of this investigation was to assess the capacity of *Agelenopsis aperta* (Gertsch) to cold-harden. This species is a funnel-web weaver of the family Agelenidae. The specimens used in this study were laboratory-reared offspring of spiders collected from desert grassland and lava bed habitats at an elevation of 1600m in south-central New Mexico. In this area winters may be severe with heavy snowfall and extended periods of sub-zero temperatures. Advanced juveniles overwinter from late October until activity resumes in March (S. E. Riechert, pers. comm.; Riechert 1974). This species population has been extensively studied with respect to habitat selection and web-site distribution by Riechert *et al.* (1973), Riechert (1974), Riechert and Tracy (1975), Riechert (1976) and Riechert and Gillespie (in press).

Second generation laboratory-reared specimens were exposed to an artificial "summer-autumn-winter cycle" by gradually exposing them to decreasing temperature during a 72 day laboratory experiment. Periodically low temperature tolerance was evaluated by determining whole body supercooling points, freeze tolerance versus susceptibility, cryoprotectant titers and rates of metabolism.

MATERIALS AND METHODS

Initial stocks of *Agelenopsis aperta* were collected from desert grasslands in south-central New Mexico, U.S.A. (Riechert *et al.* 1973). All specimens used were juvenile second-generation laboratory-reared for their entire lives at temperatures between 24 and 28°C at the University of Tennessee. Their age was similar to those of spiders overwintering in natural habitat. The spiders were mailed to the University of Houston and allowed to acclimate to 26 ± 1°C for three weeks prior to experimentation. Each week 2-3 live *Musca domestica* adults and droplets of water were provided for each spider. However, spiders were not fed for five days prior to supercooling point, cryoprotectant and respiration rate determinations. Spiders were fasted to remove potential nucleating agents in the food which might result in artifically high supercooling points.

Spiders were cold-acclimated using 5°C temperature steps decreasing from 20°C to 0°C over a 72-day period. Details of the acclimation schedule are shown in Fig. 1. Groups of 4-6 males and females (e.g. females and immature individuals) were removed periodically and tested for supercooling point, cryoprotectant levels and freeze tolerance using methods described by Lee and Baust (1981). A cooling rate of approximately 1°C/min was used for supercooling point determinations. The cryoprotectant levels were analyzed using high performance liquid chromatography with a radially compressed amine modified silica column (Hendrix *et al.* 1981, Lee *et al.* 1983). Freeze tolerance or susceptibility was assessed by allowing the body temperature of the spider to return to the temperature of its supercooling point after the release of the heat associated with the freezing of body water. Post-freezing survival was evaluated after 24 hr at 5°C.

Oxygen consumption was determined using a microrespirometry system described by Lee and Baust (1982). Each respirometer consisted of a 5cc disposable plastic syringe depressed to 2cc with a 20 μl micropipette. Two groups of eight females were acclimated for 12 days without food at 26 or 10°C prior to oxygen consumption determinations. Each spider was placed in a separate microrespirometer and equilibrated for 30 min at 15°C followed by oxygen consumption measurements during the next two hours. The

same individuals were then transferred to 10°C and respiration measurements were repeated as described above. All determinations were made between 1300-1800 hr. Oxygen consumption is expressed in nanoliters (nl) per mg live weight per hour.

RESULTS

Survival was high (> 90%) during the 72-day period of low temperature acclimation in the laboratory and did not differ between cold-acclimated spiders and ones held continuously at 26°C. Fewer males were available for study and as a result were tested only during the first 30 days of the acclimation schedule. At no time during acclimation did a spider survive freezing of body fluids. However, cooling to temperatures immediately above the supercooling point caused no apparent injury in any spider, including individuals maintained at 26°C without cold acclimation.

Supercooling points of males and females were similar and remained unchanged during the first 30 days of acclimation to low temperature (Fig. 1). The supercooling point represents the lower temperature limit for survival under natural conditions for organisms intolerant of freezing. Therefore, these data indicate that males and females have similar levels of cold tolerance. Although in most samples the supercooling point values were tightly clustered in the -8 to -12°C range, some individuals extended supercooling to as
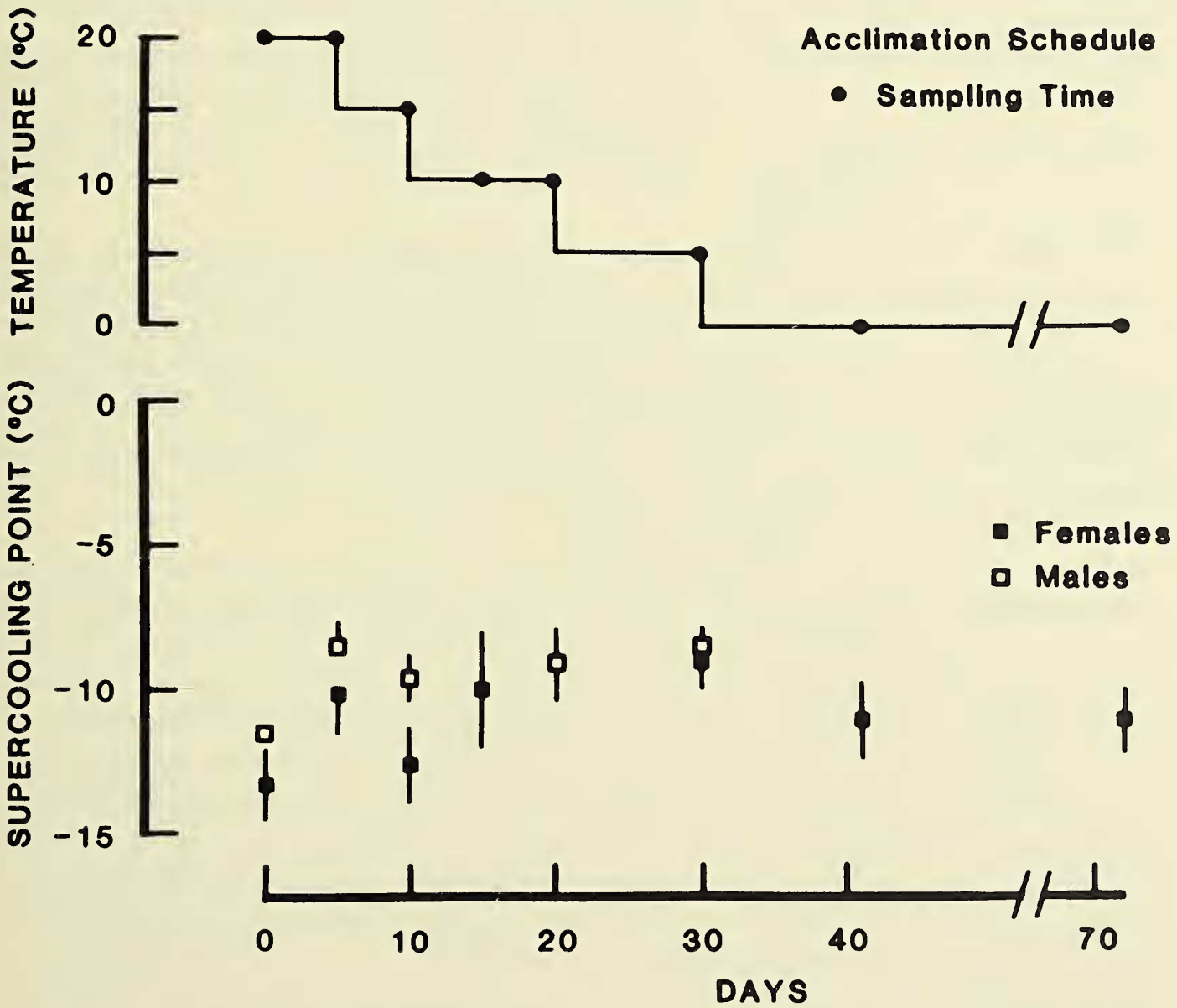

Fig. 1.–Top: Laboratory low temperature acclimation schedule for *Agelenopsis aperta*. Bottom: Comparison of supercooling points for males and females of *A. aperta* determined at intervals during the acclimation schedule (X ± SE).

low as -18.2°C before spontaneous nucleation occurred. Even after 72 days of low temperature exposure, no change in the lower limit of cold tolerance as judged by whole body supercooling points was observed (Fig. 1).

Homogenized spider extracts were analyzed using high performance liquid chromatographic techniques to determine whether low temperature acclimation had an effect on endogenous levels of cryoprotectants. Elevated levels of typical cryoprotectants including glycerol, sorbitol, trehalose, glucose, mannitol and erythritol were not detected. In fact, no glycerol, trehalose or erythritol were identified in chromatograms despite detection limits of greater than 0.05 μg per mg of live weight.

Two groups of eight female *A. aperta* were acclimated to 10 or 26°C for 12 days. Measurement of oxygen consumption at 10 or 15°C revealed no significant differences between the groups. The combined consumption rates ($\bar{x}$ ± SEM) at 10 and 15°C were, respectively, 112 ± 9 and 171 ± 15 nl/mg/hr.

DISCUSSION

Cold-hardening in spiders appears to be restricted to the avoidance of tissue freezing by depressing the whole body supercooling point. Kirchner (1973) found no evidence for freeze tolerance in studies conducted during the winter with 50 species belonging to 15 families. The data of other investigators are consistent with his observations (Table 1). Regardless of the length of cold acclimation, freeze tolerance was never observed in *A. aperta*. Cooling to temperatures immediately above the supercooling point, however, produced no apparent injury. In general it appears that spiders tolerate exposure to low temperature as long as the limit of supercooling of body fluids is not exceeded. A single exception is the tropical species, *Agelena consociata* which dies after a few hours of exposure to 0°C (Kirchner 1973).

In certain spiders considerable increases in freeze avoidance by supercooling may be observed on a seasonal basis. *Philodromus* immatures lower the supercooling point from -6.2°C in summer to -26.2°C in winter (Duman 1979). Overwintering eggs have the greatest capacity for freeze avoidance with supercooling points extending below -30°C (Table 2). In contrast, overwintering stages of some species lack specific mechanisms of changing cold-hardiness as evidenced by the similarity between summer and winter supercooling points. In Table 2 five species have summer and winter values within one degree of each other and none below -6.8°C.

Supercooling points for *A. aperta* were relatively constant regardless of the temperature or duration of low temperature exposure. Apparently this species lacks the capacity for cold-hardening despite the fact that it is exposed to sub-zero temperatures in the field. A second possibility is that low temperatures alone are not sufficient to induce hardening in this species. For example, seasonal changes in daylength provide a reliable cue for cold-hardening (Duman and Horwath 1983). This alternative is perhaps less likely since the four other agelenids listed in Table 2 apparently, also, lack the capacity for cold-hardening. For phylogenetic reasons the Agelenidae as a group may not have evolved biochemical or physiological mechanisms of seasonally enhancing low temperature tolerance. Seasonal field collections which assess cold tolerance in the natural habitat are needed to definitively resolve this question for *A. aperta*.

Physioloigcal mechanisms regulating the supercooling point are poorly understood. Although glycerol accumulation has been associated with cold-hardening in several species (Kirchner and Kestler 1969, Duman 1979) other common cryoprotectants, such as sorbitol, glucose and trehalose have not been identified in overwintering spiders.

Table 1.–Summary of cold-hardiness in overwintering spiders. Blanks in table indicate that specific information was not provided in the original article or that a given parameter was not investigated. Mean supercooling points are for spiders collected during the winter unless noted otherwise. Unless specifically stated the overwintering stage of spiders studied by Kirchner (1973) were adults or immatures. All species are intolerant of tissue freezing.

Taxon	Study Site	Overwintering Stage	Supercooling Point (°C)	Source
Agelenidae				
Agelenopsis aperta	New Mexico, U.S.A. (Lab reared)	Adult, Immature	-8.0 to -12.0	Present study
Cicurina cicurea	W. Germany		-6.7	Kirchner, 1973
Coelotes terrestris	W. Germany		-6.2 (Winter) -5.3 (Summer)	Kirchner, 1973 Kirchner, 1973
Histopona torpida	W. Germany		-6.5 (Winter) -6.0 (Summer)	Kirchner, 1973 Kirchner, 1973
Tegenaria sp.	W. Germany		-8.0	Kirchner, 1973
Amaurobiidae				
Amaurobius fenestralis	W. Germany		-6.6	Kirchner, 1973
Araneidae				
Araneus cornutus	W. Germany	Adult, Immature	-23 (Winter) -8 (Summer)	Kirchner and Kestler, 1969
Argiope aurantia	Illinois, U.S.A.	Spiderling		Riddle, 1981
Meta menardi	W. Germany		-4.0	Kirchner, 1973
Singa nitidula	W. Germany		-21.4	Kirchner, 1973
Clubionidae				
Clubiona sp. [1]	Indiana, U.S.A.	Immature	-15.4 (Winter) -9.2 (Warm-acclimated)	Duman, 1979
Clubiona phragmitis	W. Germany		-16.1	Kirchner, 1973
Eresidae				
Eresus niger	W. Germany		-16.6	Kirchner, 1973
Linyphiidae				
Bolyphantes index [1]	Norway	Adult	-15.2	Husby and Zachariassen, 1980
Floronia bucculenta	W. Germany	Egg	-30 (Summer and Winter)	Schaefer, 1976
Linyphia sp.	W. Germany	Egg	-25.2 to -33.8	Kirchner, 1973
Lycosidae				
Pardosa lugubris	W. Germany		-6.8 (Winter) -5.8 (Summer)	Kirchner, 1973
Nesticidae				
Nesticus cellulanus	W. Germany		-4.7 (Summer and Winter)	Kirchner, 1973
Philodromidae				
Philodromus sp. [1]	Indiana, U.S.A.	Immature	-26.2 (Winter) -6.2 (Summer)	Duman, 1979
Philodromus sp.	W. Germany		-21.5	Kirchner, 1973
Tetragnathidae				
Pachygnatha clercki	W. Germany		-5.8	Kirchner, 1973
Theridiidae				
Tentana castanea	W. Germany		-9.5	Kirchner, 1973
T. triangulosa	W. Germany		-10.9	Kirchner, 1973
Theridion deuticulatum	W. Germany		-11.4	Kirchner, 1973
T. notatum	W. Germany		-26.1	Kirchner, 1973
T. tepidariorum	W. Germany		-8.2	Kirchner, 1973

[1] Thermal hysteresis factors present.

Thermal-hysteresis factors are present in the hemolymph of several overwintering spiders (Duman 1979, Husby and Zachariassen 1980, Duman and Horwath 1983). These proteins produce a thermal hysteresis between the freezing and melting points and are similar in this respect to antifreeze proteins in polar fishes and some insects. Recently, Zachariassen and Husby (1982) have hyposthesized that these factors may function to enhance supercooling capacity by absorption to the surface of embryo ice nuclei and, thereby, prevent additional growth of the ice crystal. Additional studies are needed in order to determine the general significance of thermal-hysteresis factors in relation to mechanisms of cold-hardening in spiders.

Terrestrial arthropods from thermostable habitats generally lack the capacity for compensatory acclimation of respiration rate, whereas ones from variable habitats may possess considerable potential for adjustment (Hazel and Prosser 1974). Metabolic studies related to overwintering in spiders have produced varying results. A seasonal decrease in oxygen consumption has been reported for *Pisaura mirabilis* (Dondale and Legendre 1971) and *Araneus cornutus* (Kirchner 1973). This decrease may represent a state of winter diapause similar to that commonly observed in insects (Dondale and Legendre 1971). On the other hand, no evidence for seasonal acclimation of respiration rate was observed in spiderlings of *Argiope aurantia*, a species which overwinters in exposed sites above the ground (Riddle and Markezich 1981). However, laboratory studies suggest a potential for metabolic compensation since low temperature acclimation elevated the respiratory rate of spiderlings with respect to animals held at higher temperatures. Several species studied by Anderson (1970) respond to acclimation at 30°C by reducing oxygen consumption, but did not respond to low temperature acclimation at 10°C by increasing the metabolic rate. The results of our study suggest a lack of compensatory change in *A. aperta*.

The capacity for cold-hardening and the limit of cold temperature tolerance are often closely correlated with the overwintering microhabitat. Spiders which overwinter above the ground on vegetation or beneath the bark of dead standing trees possess the greatest cold tolerance (Kirchner 1973). Cave dwelling or species overwintering on the ground or in the soil are less tolerant with supercooling points between -4 and -8°C (Table 2). Furthermore, the lower lethal temperature for these species often remains essentially the same throughout the year. Edgar and Loenen (1974) suggest that the more northerly distribution of *Pardosa lugubris* relative to congeneric species is possible due to its protected hibernaculum in leaf litter.

Agelenopsis aperta is a ground dwelling species whose funnel retreat may extend 25-50cm into cracks in the substrate (Riechert *et al.* 1973). Agelenids, in general, retreat into their funnels during periods of unfavorable environmental temperature (S. E. Riechert, pers. comm.). The scorpion, *Paruroctonus aquilonalis*, also inhabits desert grasslands in New Mexico and similar to *A. aperta*, retreats into burrows in the ground (Riddle and Pugach 1976). In January with low ambient temperatures of -14°C, burrow temperatures for *P. aquilonalis* at 2 and 4 cm depth were respectively -12 and -6°C. These observations suggest that by moving only a few cm into the substrate *A. aperta* would be able to avoid environmental temperatures approaching its supercooling point.

The selection of a protected overwintering microhabitat obviates the need for seasonal mechanisms enhancing cold tolerance. The combination of a normal (i.e. non-cold-hardened) supercooling point of -8 to -10°C and movement into a moderate thermally buffered hibernaculum is likely sufficient protection for a species inhabiting southern temperature regions. In turn, this provides an explanation for the lack of mechanisms of

cold-hardening and metabolic compensation in *A. aperta* and other species overwintering in protected hibernacula.

ACKNOWLEDGMENTS

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